Poster #3172

In situ functional cell phenotyping reveals microdomain networks in colorectal cancer recurrence

Unsupervised cellular phenotypic hierarchy enables spatial intratumor heterogeneity characterization, recurrence-associated microdomains discovery, and harnesses network biology from hyperplexed in-situ fluorescence images of colorectal carcinoma

Samantha A. Furman[1], Andrew M. Stern[1,2], Shikhar Uttam[1], D. Lansing Taylor[1,2], Filippo Pullara[3], S. Chakra Chennubhotla[1,2,3]

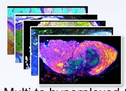
[1]Department of Computational and Systems Biology, University of Pittsburgh, PA, 15260, USA; [2]University f Pittsburgh Drug Discovery Institute, University of Pittsbugh, Pittsburgh, PA 15261, USA; [3]SpIntellx, Inc., 2425 Sidney St, Pittsburgh, PA 15203

Abstract

Tumors are dynamic ecosystems comprising localized niches, *microdomains*, possessing distinct compositions and spatial configurations of cancer and non-cancer cell populations. Microdomains determine the extent of intratumor heterogeneity, critical to disease progression and response to therapy [1]. Microdomain-specific network signaling supports the existence of a continuum of phenotypic states and the consequent emergence of functional plasticity in responding to perturbations [2, 3].

- **Problem:** Predefined cell types with binary cell states fail to capture this intrinsic functional plasticity.
- **Solution**: We present an unsupervised machine learning algorithm [4] to build a hierarchy of functional phenotypes on a continuum and combine it with our previously proposed analytical frameworks, pointwise mutual information [5] and spatial network biology [6], to discover outcome-associated microdomains.
- **Results**: This integrated approach applied to an immunofluorescence-based (51 biomarkers) image dataset of colorectal carcinoma primary tumors (N=213) from [7] discovers **recurrence-associated microdomains** visualized as distinct spatial configurations of heterogeneous phenotypic clusters.
- **Conclusions**: We find that microdomain-specific network dysregulation supporting cancer stem cell maintenance and immunosuppression appear to be necessary for driving the recurrence phenotype [8].

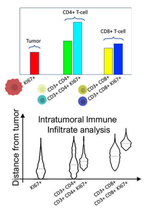
Methods



Multi to hyperplexed (>9) fluorescence or mass spec-based biomarker labeling and imaging

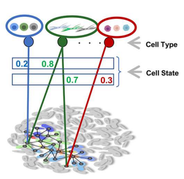
Current approaches

Functional Cell Phenotyping



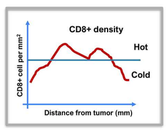
- Pre-defined cell types
- Binary (ON/OFF) cell states

Our approach

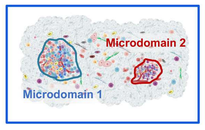


- Unbiased cell types, continuum of cell states, spatially informed
- Identify and characterize complex functionally intermediate cells

Microdomain Discovery



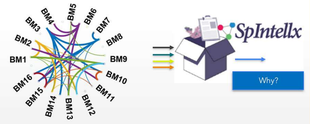
- Pre-defined microdomains (e.g. hot/cold/exhausted, etc.)
- Oversimplification of spatial intratumor heterogeneity



- Heterogeneous local tissue niches
- Distinct spatial interactions between cancer and stromal cells
- Critical to disease progression

Spatial Systems Pathology and Explainable AI

- No spatial systems pathology
- Black-box AI (end-users get no explanations)



- Patient-specific molecular targets and pathway interactions defined within microdomains driving tumor progression
- Explain why a particular recommendation is made; build trust and confidence

Results

Automated Functional Cell Phenotyping

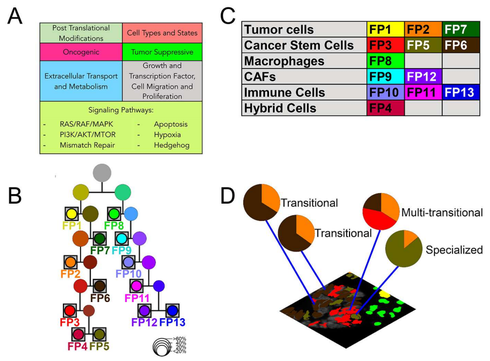
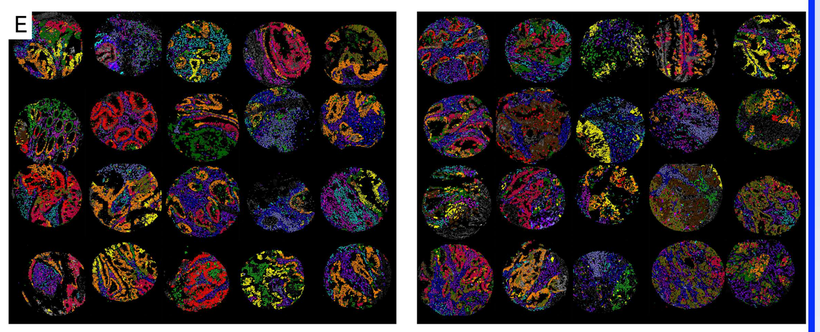


Figure 1: A. List of cellular processes covered by the biomarker panel in the CRC hyperplexed data [7]; **B**. Hierarchy of 13 distinct functional phenotypes (FP); **C**. Interpreting functional diversity, e.g., FP4 is a hybrid phenotype between macrophages and tumor cells); **D**. Specialized and non-specialized cell states. based on the FP ownership probabilities. Biologically, the non-specialized cells (transitional and multi-transitional) represent cells undergoing a transformation (e.g., epithelial-mesenchymal-transition, cell-fusion) between FPs.



E. For illustration, each cell is assigned to a distinct FP based on the highest ownership probability. Tissue samples from the outcome-based patient cohorts **NED-8yrs** and **REC-3yrs** are comprised of heterogenous populations of FPs. **We find the composition of FPs is not associated with patient outcomes (time-to-recurrence), thus motivating the discovery of spatial intratumor heterogeneity in the form of microdomains**.

Microdomain Discovery

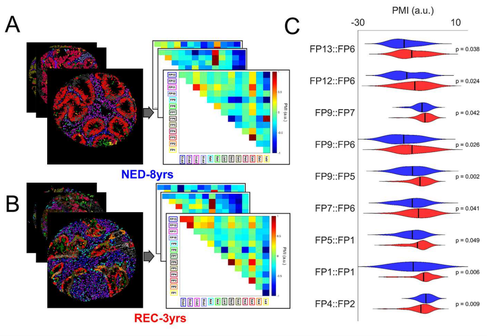
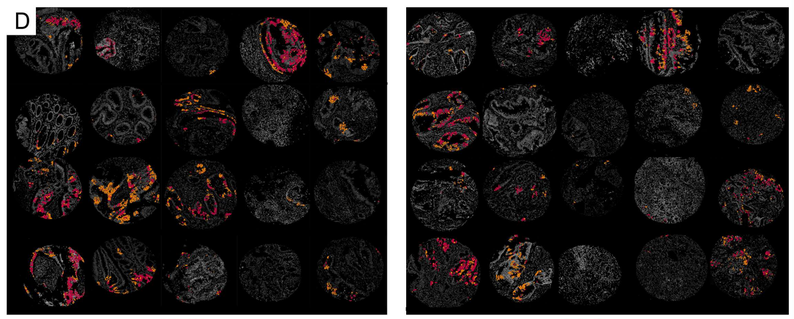


Figure 2: A and B. Pointwise mutual information (PMI) maps [6] are computed for each tissue sample from the two outcome cohorts (NED-8yrs, REC-3yrs), to quantify the spatial co-occurrence of any given FP-pair relative to a background distribution. **C.** Aggregating the two outcome-based patient cohorts we find 9 FP-pairs with statistically significant spatial co-occurrence patterns. These significant pairs form two distinct microdomains (**Figure 3A**).



D. Visualizing the difference in the spatial co-occurrence patterns of **microdomain 2** consisting of **(FP2, FP4)** between the two outcome-based patient cohorts. **Microdomain 2 spatially co-occurs more likely in the NED-8yrs cohort, thus suggesting a tumor suppressing property.**

Spatial Systems Pathology and Explainable AI

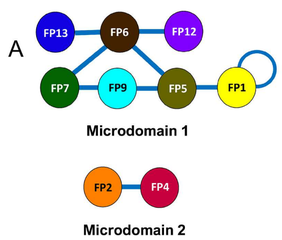
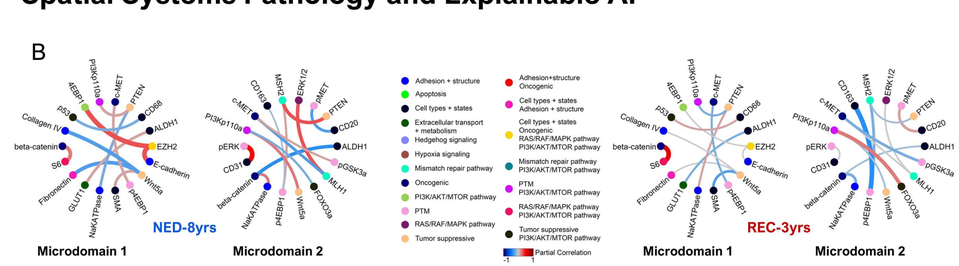


Figure 3: A. Two microdomains emerge from the FP pairwise significance analysis (**Figure 2C**): **microdomain 1** – consisting of an epithelial and stromal network between FP1, FP5, FP6, FP7, FP9, FP12, and FP13 and **microdomain 2** – consisting of an epithelial pairwise interaction between FP2 and FP4. Note: **Microdomain 1** spatially co-occurs more likely in the REC-3yrs cohort, suggesting a **tumor promoting** property; **microdomain 2** spatially co-occurs more likely in the NED-8yrs cohort, suggesting a **tumor suppressing** property



B. Within each spatial microdomain, we can identify a recurrence-associated biomarker network through a partial correlation network analysis [1]. The biomarkers are grouped based on their presumed cellular functions/processes and the thickness of each edge is coordinated to the partial correlation value between the biomarker pair. **xAI recommendations:**

- **Regulatory switch drives the recurrence phenotype: sign changes in partial correlation plus co-occurrence of cell phenotypes**.
- **Why: biomarker pairs are significantly different, many with a change in sign, when a comparison is made between the NED-8yr versus the REC-3yrs cohorts. This suggests that a distinct difference in network dysregulation in addition to co-occurrence of FPs per se is necessary for driving the recurrence phenotype.**

Discussion

- Our unsupervised and spatially informed approach enables tumor architecture to drive the discovery of a continuum of known and new cell types and states.
- This approach applied to CRC primary tumor tissue samples identified 13 unbiased FP's with heterogenous properties.
- With spatial analysis, we automatically discovered two recurrence-associated microdomains [5].
- Microdomain-specific partial correlation analysis of biomarker pairs shows a strikingly significant difference between the two patient cohorts.
- We find that within the evolving tumor microenvironment, the molecular signaling networks within each microdomain undergo a regulatory switch to confer a recurrence phenotype supported by cancer stem cell maintenance and immunosuppression
- Any level of the FP hierarchy can be used for the spatial analysis of a tumor sample. We previously reported the use of the first level of this hierarchy (epithelial and stromal domains) to successfully predict the risk of 5-year recurrence in CRC [6].
- We did a virtual simulation (results not shown) to provide evidence for performing iterative cycles of imaging and computational analysis with an optimal 15 biomarker set to fully exploit the capabilities of our analytical framework in combination with a non-destructive multi (< 9) to hyperplexed (> 9 biomarkers) imaging platforms.
- The framework presented here forms the basis of an explainable AI platform [9] with applications probing and modulating tumor environment including prognostics, diagnostics, patient stratification for clinical trials, drug target identification, and personalized therapeutic strategy optimization including immunotherapy.

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The computational and systems pathology intellectual property (US patent application 62/847,622) from this work is owned by the University of Pittsburgh and is exclusively licensed to SpIntellx Inc., Pittsburgh, PA (http://www.spintellx.com).